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<b>(54) Title:</b> FRAMEWORK MUTATED ANTIBODIES AND THEIR PREPARATION <i>Good enough</i>  <b>(57) Abstract</b> <p>An altered antibody chain is produced in which the CDR's of the variable domain of the chain are derived from a first mammalian species. The framework-encoding regions of DNA encoding the variable domain of the first species are mutated so that the mutated framework-encoding regions encode a framework derived from a second different mammalian species. The or each constant domain of the antibody chain, if present, are also derived from the second mammalian species. An antibody which is capable of binding to human CD4 antigen is also provided together with a pharmaceutical composition comprising the antibody.</p>		

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# FRAMEWORK MUTATED ANTIBODIES AND THEIR PREPARATION

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The present invention relates to altered antibodies and their preparation. The invention is typically applicable to the production of humanised antibodies.

- 5     Antibodies typically comprise two heavy chains linked together by disulphide bonds and two light chains. Each light chain is linked to a respective heavy chain by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains.
- 10   Each light chain has a variable domain at one end and a constant domain at its other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The constant
- 15   domains in the light and heavy chains are not involved directly in binding the antibody to antigen.

- The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the light and heavy chains have the same general structure and
- 20   each domain comprises a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases
- 25   forming part of, the beta-sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site.

- The preparation of an altered antibody in which the CDRs
- 30   are derived from a different species than the framework of the antibody's variable domains is disclosed in EP-A-0239400. The CDRs may be derived from a rat or mouse monoclonal antibody. The framework of the variable domains, and the constant domains, of the altered antibody
- 35   may be derived from a human antibody. Such a humanised

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antibody elicits a negligible immune response when administered to a human compared to the immune response mounted by a human against a rat or mouse antibody. Humanised CAMPATH-1 antibody is disclosed in EP-A-0328404.

5 We have now devised a new way of preparing an altered antibody. In contrast to previous proposals, this involves altering the framework of a variable domain rather than the CDRs. This approach has the advantages that it does not require a pre-existing cDNA encoding, for example, a human  
10 framework to which to reshape and that it is technically easier than prior methodologies.

Accordingly, the present invention provides a process for the preparation of an antibody chain in which the CDRs of the variable domain of the antibody chain are derived  
15 from a first mammalian species and the framework of the variable domain and, if present, the or each constant domain of the antibody chain are derived from a second different mammalian species, which process comprises:

(i) mutating the framework-encoding regions of DNA  
20 encoding a variable domain of an antibody chain of the said first species such that the mutated framework-encoding regions encode the said framework derived from the said second species; and

(ii) expressing the said antibody chain utilising the  
25 mutated DNA from step (i).

A variable domain of either or both chains of an antibody can therefore be altered by:

(a) determining the nucleotide and predicted amino acid sequence of a variable domain of a selected antibody chain  
30 of the said first species;

(b) determining the antibody framework to which the framework of the said variable domain is to be altered;

(c) mutating the framework-encoding regions of DNA encoding the said variable domain such that the mutated

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framework-encoding regions encode the framework determined upon in step (b);

- (d) linking the mutated DNA obtained in step (c) to DNA encoding a constant domain of the said second species and  
5 cloning the DNA into an expression vector; and

(e) introducing the expression vector into a compatible host cell and culturing the host cell under such conditions that antibody chain is expressed.

- The antibody chain may be co-expressed with a  
10 complementary antibody chain. At least the framework of the variable domain and the or each constant domain of the complementary chain generally are derived from the said second species also. A light chain and a heavy chain may be co-expressed. Either or both chains may have been  
15 prepared by the process of the invention. Preferably the CDRs of both chains are derived from the same selected antibody. An antibody comprising both expressed chains can be recovered.

- The antibody preferably has the structure of a natural  
20 antibody or a fragment thereof. The antibody may therefore comprise a complete antibody, a (Fab')<sub>2</sub> fragment, a Fab fragment, a light chain dimer or a heavy chain. The antibody may be an IgG such as an IgG1, IgG2, IgG3 or IgG4 IgM, IgA, IgE or IgD. Alternatively, the antibody may be a  
25 chimaeric antibody of the type described in WO 86/01533.

- A chimaeric antibody according to WO 86/01533 comprises an antigen binding region and a non-immunoglobulin region. The antigen binding region is an antibody light chain variable domain or heavy chain variable domain. Typically,  
30 the chimaeric antibody comprises both light and heavy chain variable domains. The non-immunoglobulin region is fused at its C-terminus to the antigen binding region. The non-immunoglobulin region is typically a non-immunoglobulin protein and may be an enzyme region, a region derived from  
35 a protein having known binding specificity, from a protein

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toxin or indeed from any protein expressed by a gene. The two regions of the chimaeric antibody may be connected via a cleavable linker sequence.

The invention is preferably employed to humanise an antibody, typically a monoclonal antibody and, for example, a rat or mouse antibody. The framework and constant domains of the resulting antibody are therefore human framework and constant domains whilst the CDRs of the light and/or heavy chain of the antibody are rat or mouse CDRs. Preferably all CDRs are rat or mouse CDRs. The antibody may be a human IgG such as IgG1, IgG2, IgG3, IgG4; IgM; IgA; IgE or IgD carrying rat or mouse CDRs.

The process of the invention is carried out in such a way that the resulting antibody retains the antigen binding capability of the antibody from which it is derived. An antibody is reshaped according to the invention by mutating the framework-encoding regions of DNA coding for the variable domains of the antibody. This antibody and the reshaped antibody should both be capable of binding to the same antigen.

The starting antibody is typically an antibody of a selected specificity. In order to ensure that this specificity is retained, the variable domain framework of the antibody is preferably reshaped to about the closest variable domain framework of an antibody of another species. By "about the closest" is meant about the most homologous in terms of amino acid sequences. Preferably there is a homology of at least 50% between the two variable domains.

There are four general steps to reshape a monoclonal antibody. These are:

(1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy chain variable domains;

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(2) designing the reshaped antibody, i.e. deciding which antibody framework region to use during the reshaping process;

(3) the actual reshaping methodologies/techniques; and

5 (4) the transfection and expression of the reshaped antibody.

These four steps are explained below in the context of humanising an antibody. However, they may equally well be applied when reshaping to an antibody of a non-human  
10 species.

Step 1: Determining the nucleotide and predicted amino acid sequence of the antibody light and heavy chain variable domains

To reshape an antibody only the amino acid sequence of  
15 antibody's heavy and light chain variable domains needs to be known. The sequence of the constant domains is irrelevant because these do not contribute to the reshaping strategy. The simplest method of determining an antibody's variable domain amino acid sequence is from cloned cDNA  
20 encoding the heavy and light chain variable domain.

There are two general methods for cloning a given antibody's heavy and light chain variable domain cDNAs: (1) via a conventional cDNA library, or (2) via the polymerase chain reaction (PCR). Both of these methods are widely  
25 known. Given the nucleotide sequence of the cDNAs, it is a simple matter to translate this information into the predicted amino acid sequence of the antibody variable domains.

30 Step 2: Designing the reshaped antibody

There are several factors to consider in deciding which human antibody sequence to use during the reshaping. The reshaping of light and heavy chains are considered independently of one another, but the reasoning is  
35 basically similar for each.

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This selection process is based on the following rationale: A given antibody's antigen specificity and affinity is primarily determined by the amino acid sequence of the variable region CDRs. Variable domain framework residues have little or no direct contribution. The primary function of the framework regions is to hold the CDRs in their proper spacial orientation to recognize antigen. Thus the substitution of rodent CDRs into a human variable domain framework is most likely to result in retention of their correct spacial orientation if the human variable domain is highly homologous to the rodent variable domain from which they originated. A human variable domain should preferably be chosen therefore that is highly homologous to the rodent variable domain(s).

15 A suitable human antibody variable domain sequence can be selected as follows:

1. Using a computer program, search all available protein (and DNA) databases for those human antibody variable domain sequences that are most homologous to the rodent antibody variable domains. This can be easily accomplished with a program called FASTA but other suitable programs are available. The output of a suitable program is a list of sequences most homologous to the rodent antibody, the percent homology to each sequence, and an alignment of each sequence to the rodent sequence. This is done independently for both the heavy and light chain variable domain sequences. The above analyses are more easily accomplished if customized sub-databases are first created that only include human immunoglobulin sequences. This has two benefits. First, the actual computational time is greatly reduced because analyses are restricted to only those sequences of interest rather than all the sequences in the databases. The second benefit is that, by restricting analyses to only human immunoglobulin

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sequences, the output will not be cluttered by the presence of rodent immunoglobulin sequences. There are far more rodent immunoglobulin sequences in databases than there are human.

- 5 2. List the human antibody variable domain sequences that have the most overall homology to the rodent antibody variable domain (from above). Do not make a distinction between homology within the framework regions and CDRs. Consider the overall homology.
- 10 3. Eliminate from consideration those human sequences that have CDRs that are a different length than those of the rodent CDRs. This rule does not apply to CDR 3, because the length of this CDR is normally quite variable. Also, there are sometimes no or very few human sequences  
15 that have the same CDR lengths as that of the rodent antibody. If this is the case, this rule can be loosened, and human sequences with one or more differences in CDR length can be allowed.
- 20 4. From the remaining human variable domains, the one is selected that is most homologous to that of the rodent.
5. The actual reshaped antibody (the end result) should contain CDRs derived from the rodent antibody and a variable domain framework from the human antibody chosen above.

25 Step 3: The actual reshaping methodologies/techniques

- A cDNA encoding the desired reshaped antibody is preferably made beginning with the rodent cDNA from which the rodent antibody variable domain sequence(s) was originally determined. The rodent variable domain amino  
30 acid sequence is compared to that of the chosen human antibody variable domain sequence. The residues in the rodent variable domain framework are marked that need to be changed to the corresponding residue in the human to make the rodent framework identical to that of the human

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framework. There may also be residues that need adding to or deleting from the rodent framework sequence to make it identical to that of the human.

Oligonucleotides are synthesised that can be used to  
5 mutagenize the rodent variable domain framework to contain the desired residues. Those oligonucleotides can be of any convenient size. One is normally only limited in length by the capabilities of the particular synthesizer one has available. The method of oligonucleotide-directed in vitro  
10 mutagenesis is well known.

The advantages of this method of reshaping as opposed to splicing CDRs into a human framework are that (1) this method does not require a pre-existing cDNA encoding the human framework to which to reshape and (2) splicing CDRs  
15 is technically more difficult because there is usually a large region of poor homology between the mutagenic oligonucleotide and the human antibody variable domain. This is not so much a problem with the method of splicing human framework residues onto a rodent variable domain because  
20 there is no need for a pre-existing cDNA encoding the human variable domain. The method starts instead with the rodent cDNA sequence. Also, splicing framework regions is technically easier because there is a high degree of homology between the mutagenic oligonucleotide and the  
25 rodent variable domain framework. This is true because a human antibody variable domain framework has been selected that is most homologous to that of the rodent.

The advantage of the present method of reshaping as opposed to synthesizing the entire reshaped version from  
30 scratch is that it is technically easier. Synthesizing a reshaped variable domain from scratch requires several more oligonucleotides, several days more work, and technical difficulties are more likely to arise.

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Step 4: The transfection and expression of the reshaped antibody

Following the mutagenesis reactions to reshape the antibody, the cDNAs are linked to the appropriate DNA  
5 encoding light or heavy chain constant region, cloned into an expression vector, and transfected into mammalian cells. These steps can be carried out in routine fashion. A reshaped antibody may therefore be prepared by a process comprising:

- 10 a) preparing a first replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least a variable domain of an Ig heavy or light chain, the variable domain comprising framework regions from a first antibody and CDRs comprising  
15 at least parts of the CDRs from a second antibody of different specificity;
- b) if necessary, preparing a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the  
20 variable domain of a complementary Ig light or heavy chain respectively;
- c) transforming a cell line with the first or both prepared vectors; and
- d) culturing said transformed cell line to produce  
25 said altered antibody.

Preferably the DNA sequence in step a) encodes both the variable domain and the or each constant domain of the antibody chain, the or each constant domain being derived from the first antibody. The antibody can be recovered and  
30 purified. The cell line which is transformed to produce the altered antibody may be a Chinese Hamster Ovary (CHO) cell line or an immortalised mammalian cell line, which is advantageously of lymphoid origin, such as a myeloma, hybridoma, trioma or quadroma cell line. The cell line may  
35 also comprise a normal lymphoid cell, such as a B-cell,

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which has been immortalised by transformation with a virus, such as the Epstein-Barr virus. Most preferably, the immortalised cell line is a myeloma cell line or a derivative thereof.

5     Although the cell line used to produce the altered antibody is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a yeast cell line, may alternatively be used. In particular, it is envisaged that E. coli - derived bacterial strains  
10    could be used.

It is known that some immortalised lymphoid cell lines, such as myeloma cell lines, in their normal state secrete isolated Ig light or heavy chains. If such a cell line is transformed with the vector prepared in step (a) it will  
15    not be necessary to carry out step (b) of the process, provided that the normally secreted chain is complementary to the variable domain of the Ig chain encoded by the vector prepared in step (a).

However, where the immortalised cell line does not  
20    secrete or does not secrete a complementary chain, it will be necessary to carry out step (b). This step may be carried out by further manipulating the vector produced in step (a) so that this vector encodes not only the variable domain of an altered antibody light or heavy chain, but  
25    also the complementary variable domain.

Alternatively, step (b) is carried out by preparing a second vector which is used to transform the immortalised cell line. This alternative leads to easier construct preparation, but may be less preferred than the first  
30    alternative in that it may not lead to as efficient production of antibody.

In the case where the immortalised cell line secretes a complementary light or heavy chain, the transformed cell line may be produced for example by transforming a suitable  
35    bacterial cell with the vector and then fusing the

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bacterial cell with the immortalised cell line by spheroplast fusion. Alternatively, the DNA may be directly introduced into the immortalised cell line by electroporation or other suitable method.

- 5 An antibody is consequently produced in which CDRs of a variable domain of an antibody chain are homologous with the corresponding CDRs of an antibody of a first mammalian species and in which the framework of the variable domain and the constant domains of the antibody are homologous
- 10 with the corresponding framework and constant domains of an antibody of a second, different, mammalian species. Typically, all three CDRs of the variable domain of a light or heavy chain are derived from the first species.

- The present process has been applied to obtain an
- 15 antibody against human CD4 antigen. Accordingly, the invention also provides an antibody which is capable of binding to human CD4 antigen, in which the CDRs of the light chain of the antibody have the amino acid sequences:

CDR1: LASEDIYSDLA

20 CDR2: NTDTLQN

CDR3: QQYNNYPWT,

in which the CDRs of the heavy chain of the antibody have the amino acid sequences:

CDR1: NYGMA

25 CDR2: TISHDGSDTYFRDSVKG

CDR3: QGTIAGIRH, and

in which the framework of the variable domain and, if present, the or each constant domain of each chain are derived from a mammalian non-rat species.

- 30 The antibody preferably has the structure of a natural antibody or a fragment thereof. The antibody may therefore comprise a complete antibody, a (Fab')<sub>2</sub> fragment, a Fab fragment, a light chain dimer or a heavy chain.

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The antibody may be an IgG such as IgG1, IgG2, IgG3 or IgG4 IgM, IgA, IgE or IgD. Alternatively, the antibody may be a chimaeric antibody of the type described in WO 86/01533.

5 A chimaeric antibody according to WO 86/01533 comprises an antigen binding region and a non-immunoglobulin region. The antigen binding region is an antibody light chain variable domain or heavy chain variable domain. Typically the chimaeric antibody comprises both light and heavy chain  
10 variable domains. The non-immunoglobulin region is fused at its C-terminus to the antigen binding region. The non-immunoglobulin region is typically a non-immunoglobulin protein and may be an enzyme region, a region derived from a protein having known binding specificity, from a protein  
15 toxin or indeed from any protein expressed by a gene. The two regions of the chimaeric antibody may be connected via a cleavable linker sequence.

The invention is preferably employed to humanise a CD4 antibody such as a rat or mouse CD4 antibody. The  
20 framework and the constant domains of the resulting antibody are therefore human framework and constant domains whilst the CDRs of the light and/or heavy chain of the antibody are rat or mouse CDRs. Preferably all CDRs are rat or mouse CDRs. The antibody may be a human IgG such as  
25 IgG1, IgG2, IgG3, IgG4; IgM; IgA; IgE or IgD carrying rat or mouse CDRs.

Preferably the framework of the antibody heavy chain is homologous to the corresponding framework of the human antibody KOL (Schmidt et al, Hoppe-Seyler's Z. Physiol.  
30 Chem., 364 713-747, 1983). The sixth residue of framework 4 in this case is suitably Thr or Pro, preferably Thr. This residue is the 121st residue in the KOL antibody heavy chain variable region (Schmidt et al, 1983), and is identified as residue 108 by Kabat (Kabat et al, "Sequences  
35 of proteins of immunological interest", US Dept of Health

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and Human Services, US Government Printing Office, 1987). Alternatively, the framework of the antibody heavy chain is homologous to the corresponding framework of the human antibody NEW (Saul et al, J. Biol.Chem. 253: 585-597,

- 5 1978). The final residue of framework 1 in this case is suitably Ser or Thr, preferably Ser. This residue is at position 30 (Kabat et al, 1987). Preferably the framework of the antibody light chain is homologous to the variable domain framework of the protein REI (Epp et al, Eur. J. Biochem., 45, 513-524, 1974).

- The framework regions of one or both chains of a CD4 antibody can be reshaped by the present process. Alternatively, one or both chains of a CD4 antibody may be reshaped by the procedure described in EP-A-0239400. The procedure of EP-A-0239400 involves replacing CDRs rather than the replacement of frameworks. The CDRs are grafted onto a framework derived from a mammalian non-rat species, typically a human. This may be achieved by oligonucleotide-directed in vitro mutagenesis of the CDR-encoding regions of an antibody chain, light or heavy, from a mammalian non-rat species. The oligonucleotides in such an instance are selected so that the resulting CDR-grafted antibody has the light chain CDRs 1 to 3 and the heavy chain CDRs 1 to 3 shown above.

- 25 The reshaped CD4 antibody can be used to induce tolerance to an antigen. It can be used to alleviate autoimmune diseases such as rheumatoid arthritis. It can be used to prevent graft rejection. Tolerance to a graft such as an organ graft or a bone marrow transplantation can be achieved. Also, the reshaped CD4 antibody might be used to alleviate allergies. Tolerance to allergens could be achieved.

The CD4 antibody may be depleting or non-depleting. A depleting antibody is an antibody which depletes more than

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50%, for example from 90 to 99%, of target cells in vivo. A non-depleting antibody depletes fewer than 50%, for example, from 10 to 25% and preferably less than 10% of target cells in vivo. A CD4 antibody may be administered  
5 alone or may be co-administered with a non-depleting or depleting CD8 antibody. The CD4 antibody, depleting or non-depleting, and CD8 monoclonal antibody, depleting or non-depleting, may be administered sequentially in any order or may be administered simultaneously. An additional  
10 antibody, drug or protein may be administered before, during or after administration of the antibodies.

A CD4 antibody and, indeed, a CD8 antibody as appropriate are given parenterally, for example intravenously. The antibody may be administered by  
15 injection or by infusion. For this purpose the antibody is formulated in a pharmaceutical composition further comprising a pharmaceutically acceptable carrier or diluent. Any appropriate carrier or diluent may be employed, for example phosphate-buffered saline solution.

20 The amount of non-depleting or depleting CD4 and, if desired, CD8 antibody administered to a patient depends upon a variety of factors including the age and weight of a patient, the condition which is being treated and the antigen(s) to which it is desired to induce tolerance. In  
25 a model mouse system from 1 $\mu$ g to 2mg, preferably from 400 $\mu$ g to 1mg, of a mAb is administered at any one time. In humans from 3 to 500mg, for example from 5 to 200mg, of antibody may be administered at any one time. Many such doses may be given over a period of several weeks,  
30 typically 3 weeks.

A foreign antigen(s) to which it is desired to induce tolerance can be administered to a host before, during, or after a course of CD4 antibody (depleting or non-depleting) and/or CD8 antibody (depleting or non-depleting).  
35 Typically, however, the antigen(s) is administered one week

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after commencement of antibody administration, and is terminated three weeks before the last antibody administration.

Tolerance can therefore be induced to an antigen in a host by administering non-depleting or depleting CD4 and CD8 mAbs and, under cover of the mAbs, the antigen. A patient may be operated on surgically under cover of the non-depleting or depleting CD4 and CD8 mAbs to be given a tissue transplant such as an organ graft or a bone marrow transplant. Also, tolerance may be induced to an antigen already possessed by a subject. Long term specific tolerance can be induced to a self antigen or antigens in order to treat autoimmune disease such as multiple sclerosis or rheumatoid arthritis. The condition of a patient suffering from autoimmune disease can therefore be alleviated.

The following Example illustrates the invention. In the accompanying drawings:

Figure 1: shows the nucleotide and predicted amino acid sequence of rat CD4 antibody light chain variable region. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. Base pairs 1-269 (HindIII-PvuII) and 577-620 ([BglII/BclI]-BamHI) are part of the vector M13V<sub>K</sub>PCR3, while base pairs 270-576 are from the PCR product of the CD4 antibody light chain variable region (V<sub>L</sub>). CDRs (boxes) were identified by comparison to known immunological sequences (Kabat et al, "Sequences of proteins of immunological interest, US Dept of Health and Human Services, US Government Printing Office, 1987).

Figure 2: shows the nucleotide and predicted amino acid sequence of the reshaped CAMPATH-1 antibody light chain cDNA. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

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Figure 3: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody light chain cDNA CD4V<sub>L</sub>REI. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 4: shows the nucleotide and predicted amino acid sequence of rat CD4 antibody heavy chain variable region. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes. Base pairs 1-272 (HindIII-PstI) and 603-817 (BstEII-BamHI) are part of the vector M13V<sub>H</sub>PCR1, while base pairs 273-602 are from the PCR product of the CD4 antibody heavy chain variable region (V<sub>H</sub>).

Figure 5: shows the nucleotide and predicted amino acid sequence of the reshaped CAMPATH-1 antibody heavy chain cDNA. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 6: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain cDNA CD4V<sub>H</sub>NEW-Thr<sup>30</sup>. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 7: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain cDNA CD4V<sub>H</sub>NEW-Ser<sup>30</sup>. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 8: shows the heavy chain variable (V) region amino acid sequence of the human myeloma protein KOL. CDRs are identified by boxes. This sequence is taken from the Swiss-Prot protein sequence database.

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Figure 9: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region CD4V<sub>H</sub>KOL-Pro<sup>113</sup>. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 10: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region CD4V<sub>H</sub>KOL-Pro<sup>113</sup> without immunoglobulin promoter. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 11: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region CD4V<sub>H</sub>KOL-Thr<sup>113</sup>. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 12: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region CD4V<sub>H</sub>KOL-Thr<sup>113</sup> without immunoglobulin promoter. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 13: shows the results of an ELISA that compares the avidity of YNB46.1.8 and CD4V<sub>H</sub>KOL-Thr<sup>113</sup> antibodies. The X-axis indicates the concentration ( $\mu$ g/ml) of YNB46.1.8 (triangles) or CD4V<sub>H</sub>KOL-Thr<sup>113</sup> (circles) antibody. The Y-axis indicates the optical density at 492 nanometers.

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EXAMPLE1. MATERIALS AND METHODS

Isolation of monoclonal antibody. The rat-derived anti-human CD4 antibody, clone YNB46.1.8 (IgG<sub>2b</sub>, kappa light chain serotype), was the result of fusion between a rat splenocyte and the Lou strain rat myeloma cell line Y3-Ag 1.2.3 (Galfre *et al*, Nature, 277: 131-133, 1979) and was selected by its binding to a rat T cell line NB2-6TG stably transfected with an expression vector containing a complementary DNA (cDNA) encoding the human CD4 antigen (Madden *et al*, Cell, 42: 93-104, 1985). Antibody was purified by high pressure liquid chromatography (HPLC).

Isolation of Antibody Variable Regions. cDNAs encoding the V<sub>L</sub> and V<sub>H</sub> regions of the CD4 antibody were isolated by a polymerase chain reaction (PCR)-based method (Orlandi *et al*, PNAS USA, 86: 3833-3837, 1989) with some modifications. Total RNA was isolated from hybridoma cells by the guanidine thiocyanate method (Chirgwin *et al*, Biochemistry, 18: 5294, 1979), and poly(A)<sup>+</sup> RNA was isolated by passage of total RNA through and elution from an oligo(dT)-cellulose column (Aviv and Leder PNAS USA 69: 1408, 1972). Poly(A)<sup>+</sup> RNA was heated at 70°C for 5 minutes and cooled on ice just prior to use. A 25μl first strand synthesis reaction consisted of 5μg poly(A)<sup>+</sup> RNA, 250 μM each dNTP, 50 mM Tris.HCl (pH 8.2 at 42°C), 10 mM MgCl<sub>2</sub>, 100 mM KCl, 10 mM dithiothreitol, 23 units reverse transcriptase (Anglian Biotec, Colchester, U.K.), 3.5 pmoles of the V<sub>L</sub> region-specific oligonucleotide primer V<sub>K</sub>1FOR [5'-d(GTT AGA TCT CCA GCT TGG TCC C)] or the V<sub>H</sub> region-specific primer V<sub>H</sub>1FOR-B [5,-d(TGA GGA GAC GGT GAC CGT GGT CCC TTG GCC)], and incubated for 5 minutes at 20°C and then 90 minutes at 42°C.

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Subsequent 50  $\mu$ l PCR amplifications consisted of 5  $\mu$ l of the first strand synthesis reaction (unpurified), 500  $\mu$ M each dNTP, 67 mM Tris-HCl (pH 8.8 at 25°C), 17 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 10 mM MgCl<sub>2</sub>, 20  $\mu$ g/ml gelatin, 5 units TAQ DNA polymerase (Koch-Light, Haverhill, U.K.), and 25 pmoles (each) of primers V<sub>K</sub>LFOR and V<sub>K</sub>LBACK [5'-d(GAC ATT CAG CTG ACC CAG TCT CCA)] for the V<sub>L</sub> region or V<sub>H</sub>LFOR-B and the mixed primer V<sub>H</sub>LBACK [5'-d(AG GT(CG) (CA)A(GA) CTG CAG (GC)AG TC(TA) GG)] for the V<sub>H</sub> region. Reactions were overlaid with mineral oil and subjected to 30 cycles of 1.5 minutes at 95°C (denaturation), 1.5 minutes at 37°C (V<sub>L</sub>) or 50°C (V<sub>H</sub>; annealing), and 3 minutes at 72°C (extension) with a Techne PHC-1 programmable cyclic reactor. The final cycle contained a 10 minute extension time.

The samples were frozen at -20°C and the mineral oil (a viscous liquid at -20°C) was removed by aspiration. The aqueous phases were thawed, and PCR products were purified by electrophoresis in 2% agarose gels, and then double digested with either PvuII and BglIII (V<sub>L</sub>) or PstI and BstEII (V<sub>H</sub>) restriction enzymes, and cloned into the PvuII and BclI restriction sites of the vector M13V<sub>K</sub>PCR3 (for V<sub>L</sub> region; Orlandi *et al*, 1989) or the PstI and BstEII restriction sites of the vector M13V<sub>H</sub>PCR1 (for V<sub>H</sub> region). As described in the results, V<sub>L</sub> region clones were first screened by hybridisation to a <sup>32</sup>P-labeled oligonucleotide probe [5'-d(GTT TCA TAA TAT TGG AGA CA)] specific for the CDR2 of the Y3-Ag 1.2.3 V<sub>L</sub> region. V<sub>L</sub> region clones not hybridising to this probe and V<sub>H</sub> region clones were sequenced by the dideoxy chain termination method (Sanger *et al*, PNAS USA 74: 5463, 1977).

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Reshaped Light Chain Variable Region and Expression Vector Construct.

The reshaped light chain was constructed by oligonucleotide-directed in vitro mutagenesis in an M13 vector by priming with three oligonucleotides simultaneously on a 748 base single-stranded cDNA template encoding the entire  $V_L$  and kappa constant ( $C_K$ ) regions of the reshaped CAMPATH-1 antibody (Reichmann et al, Nature 332: 323-327, 1988). The three oligonucleotides [5'-d(AGA GTG ACC ATC ACC TGT CTA GCA AGT GAG GAC ATT TAC AGT GAT TTA GCA TGG TAC CAG CAG AAG CCA), 5'-d(CTG CTG ATC TAC AAT ACA GAT ACC TTG CAA AAT GGT GTG CCA AGC AGA TTC), 5'-d(ATC GCC ACC TAC TAC TGC CAA CAG TAT AAC AAT TAT CCG TGG ACG TTC GGC CAA GGG ACC)] were designed to replace each of the three CDRs in the REI-based human antibody  $V_L$  region framework that is part of the reshaped CAMPATH-1 antibody  $V_L$  region (Reichmann et al, 1988). A clone containing each of the three mutant oligonucleotides was identified by nucleotide sequencing and was subcloned into the HindIII site of the expression vector pH $\beta$ APr-1 (Gunning et al, PNAS, 84: 4831-4835, 1987) which also contained a dihydrofolate reductase gene (Ringold et al, J.Mol.Appl. Genet. 1: 165-175, 1981) driven by a truncated SV40 promoter.

Reshaped Heavy Chain Variable Regions Based on the Variable Region Framework of the Human Antibody NEW, and Expression Vector Constructs.

Two versions of the NEW-based reshaped heavy chain were created, CD4V<sub>H</sub>NEW-Thr<sup>30</sup> and CD4V<sub>H</sub>NEW-Ser<sup>30</sup>. The CD4V<sub>H</sub>NEW-Thr<sup>30</sup> version (Figure 6) encodes a threonine residue at position 30 while the CD4V<sub>H</sub>NEW-Ser<sup>30</sup> version (Figure 7) encodes a Ser residue at position 30. As a matter of convenience, CD4V<sub>H</sub>NEW-Thr<sup>30</sup> was created first by oligonucleotide-directed in vitro mutagenesis in the vector M13mp18 by priming with three oligonucleotides

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simultaneously on a 1467 base single-stranded cDNA template (Figure 5) encoding the entire heavy chain of the reshaped CAMPATH-1 antibody (Reichmann et al, 1988). The three oligonucleotides [5'-d(TCT GGC TTC ACC TTC ACC AAC TAT GGC ATG GCC TGG GTG AGA CAG CCA CCT), 5'-d(GGT CTT GAG TGG ATT GGA ACC ATT AGT CAT GAT GGT AGT GAC ACT TAC TTT CGA GAC TCT GTG AAG GGG AGA GTG), 5'-d(GTC TAT TAT TGT GCA AGA CAA GGC ACT ATA GCT GGT ATA CGT CAC TGG GGT CAA GGC AGC CTC)] were designed to replace each of the three complementarity determining regions (CDRs) in the NEW-based V<sub>H</sub> region that is part of the reshaped CAMPATH-1 antibody (Reichmann et al, 1988). A clone (Figure 6) containing each of the three mutant oligonucleotides was identified by nucleotide sequencing. CD4V<sub>H</sub>NEW-Ser<sup>30</sup> was created second by oligonucleotide-directed in vitro mutagenesis in the vector M13mp18 by priming with a single oligonucleotide on the 1458 base single-stranded cDNA template (Figure 6) encoding CD4V<sub>H</sub>NEW-Thr<sup>30</sup>. The oligonucleotide [5'-d(GCT TCA CCT TCA GCA ACT ATG GCA T)] was designed to mutate the residue at position 30 from threonine [ACC] to serine [AGC]. A clone (Figure 7) containing this mutant oligonucleotide was identified by nucleotide sequencing. Double-stranded forms of the clones CD4V<sub>H</sub>NEW-Thr<sup>30</sup> and CD4V<sub>H</sub>NEW-Ser<sup>30</sup> were subcloned as HindIII fragments into the HindIII site of the expression vector pNH316. The vector pNH316 is a modified version of the vector pH<sub>8</sub>APr-1 (Gunning et al, PNAS, 84: 4831-4835, 1987) which was engineered to contain a neomycin resistance gene driven by a metallothionine promoter.

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Reshaped Heavy Chain Variable Regions Based on the  
Variable Region Framework of the Human Antibody KOL, and  
Expression Vector Constructs

Two versions of the KOL-based reshaped heavy chain were  
5 created, CD4V<sub>H</sub>KOL-Thr<sup>113</sup> and CD4V<sub>H</sub>KOL-Pro<sup>113</sup>. The  
CD4V<sub>H</sub>KOL-Thr<sup>113</sup> version encodes a threonine residue at  
position 113 (Figure 11) while the CD4V<sub>H</sub>KOL-Pro<sup>113</sup> version  
encodes a proline residue at position 113 (Figure 9). As a  
matter of convenience, CD4V<sub>H</sub>KOL-Thr<sup>113</sup> was created first by  
10 oligonucleotide-directed in vitro mutagenesis of single-  
stranded DNA template containing the 817 base HindIII-BamHI  
fragment encoding the V<sub>H</sub> region of the rat CD4 antibody  
(Figure 4) cloned into M13mp18 by priming simultaneously  
with five oligonucleotides [5'-d(CAC TCC CAG GTC CAA CTG  
15 GTG GAG TCT GGT GGA GGC GTG GTG CAG CCT GG), 5'-d(AAG GTC  
CCT GAG ACT CTC CTG TTC CTC CTC TGG ATT CAT CTT CAG TAA CTA  
TGG CAT G), 5'-d(GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG  
TGG), 5'-d(ACT ATC TCC AGA GAT AAT AGC AAA AAC ACC CTA TTC  
CTG CAA ATG G), 5'-d(ACA GTC TGA GGC CCG AGG ACA CGG GCG  
20 TGT ATT TCT GTG CAA GAC AAG GGA C)] which were designed to  
replace the rat framework regions with the human framework  
regions of KOL. A clone containing each of the five mutant  
oligonucleotides was identified by nucleotide sequencing.  
CD4V<sub>H</sub>KOL-Pro<sup>113</sup> was created second by oligonucleotide-  
25 directed in vitro mutagenesis of single-stranded DNA  
template containing the 817 base HindIII-BamHI fragment  
encoding CD4V<sub>H</sub>KOL-Thr<sup>113</sup> cloned into M13mp18 by priming  
with the oligonucleotide [5'-d(TGG GGC CAA GGC ACC CCC GTC  
ACC GTC TCC TCA)]. A clone containing this mutant  
30 oligonucleotide was identified by nucleotide sequencing.

The immunoglobulin promoters were removed from the  
double-stranded DNA forms of clones encoding CD4V<sub>H</sub>KOL-  
Thr<sup>113</sup> (Figure 11) and CD4V<sub>H</sub>KOL-Pro<sup>113</sup> (Figure 9) by  
replacing (for both versions) the first 125 bp (HindIII-  
35 NcoI) with a HindIII-NcoI oligonucleotide linker fragment

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[5'-d(AGC TTT ACA GTT ACT GAG CAC ACA GGA CCT CAC) and its overlapping complement 5'-d(CAT GGT GAG GTC CTG TGT GCT CAG TAA CTG TAA)]. The resultant clones, CD4V<sub>H</sub>KOL-Thr<sup>113</sup> (Figure 12) and CD4V<sub>H</sub>KOL-Pro<sup>113</sup> (Figure 10), now 731 bp  
5 HindIII-BamHI fragments, were separately subcloned into the HindIII and BamHI cloning sites of the expression vector pH $\beta$ APr-1-gpt (Gunning *et al*, PNAS USA 76, 1373, 1987) into which had been cloned the human IgG1 constant region gene (Bruggemann *et al*, J.Exp.Med. 166, 1351-1361, 1987) at the  
10 BamHI site. Thus, when transfected and expressed as antibody heavy chains (see below), these reshaped V<sub>H</sub> regions are linked to human IgG1 constant regions.

#### Fluorescence activated cell sorter (FACS) analysis

The relative affinities of the reshaped antibodies to  
15 bind the CD4 antigen were estimated by FACS analysis. The CD4-expressing cells used in this analysis were a cloned rat T cell line NB2-6TG stably transfected with an expression vector containing a complementary DNA (cDNA) encoding the human CD4 antigen (Maddon *et al*, Cell, 42, 93-  
20 104, 1985). Cells were stained with the appropriate reshaped antibody followed by fluorescein-conjugated sheep anti-human antibodies (Binding Site Ltd., Birmingham, UK). Control staining (see Table 1) consisted of no antibody present during the first stage of cell staining. Mean  
25 cellular fluorescence was determined with an Ortho FACS.

#### Antibody avidity analysis

The relative avidities of the rat YNB46.1.8 antibody and the reshaped CD4V<sub>H</sub>KOL-Thr<sup>113</sup> antibody were estimated by an enzyme-linked immunosorbent assay (ELISA). Microtiter  
30 plates were coated with soluble recombinant CD4 antigen (Byrn *et al*, Nature, 344: 667-670, 1990) at 50 ul/well, 10 ug/ml, and then blocked with 100 ul/well phosphate buffered saline (PBS) containing 1.0% bovine serum albumin

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(BSA). Antibodies were diluted in PBS containing 0.1% BSA, and added to wells (50 ul/well) for 45 minutes at room temperature. Biotinylated CD4V<sub>H</sub>KOL-Thr<sup>113</sup> antibody (10 ul/well; 20 ug/ml final concentration) was then added to  
5 each well for an additional 45 minutes. Wells were washed with PBS containing 0.1% BSA, and then 50 ul streptavidin-biotinylated horseradish peroxidase complex (Amersham; Aylesbury, UK) diluted 1:1,000 was added to each well for 30 minutes. Wells were washed with PBS containing 0.1%  
10 BSA, and 100 ul substrate (25 mM citric acid, 50 mM disodium hydrogen phosphate, 0.1% (w/v) o-phenylene diamine, 0.04% (v/v) 30% hydrogen peroxide) was added to each well. Reactions were stopped by the addition of 50 ul/well 1.0 M sulfuric acid. Optical densities at 492  
15 nanometers (OD<sub>492</sub>) were determined with an ELISA plate reader.

#### Transfections.

Dihydrofolate reductase deficient chinese hamster ovary (CHO<sup>DHFR</sup>-) cells (10<sup>6</sup>/T-75 flask) were cotransfected as  
20 described (Wigler *et al*, PNAS USA 76, 1373, 1979) with 9µg of heavy chain construct and 1 µg of the light chain construct. Transfectants were selected in medium containing 5% dialysed foetal bovine serum for 2 to 3 weeks, and antibody-secreting clones were identified by  
25 ELISAs of conditioned media. Antibody was concentrated and purified by protein-A Sepharose (Trade Mark) column chromatography.

## 2. RESULTS

### Cloning of Light and Heavy Chain Variable Region cDNAs.

30 cDNAs encoding the V<sub>L</sub> and V<sub>H</sub> regions from CD4 antibody-secreting hybridoma cells were isolated by PCR using primers which amplify the segment of mRNA encoding the N-

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terminal region through to the J region (Orlandi *et al*, 1989).  $V_L$  and  $V_H$  region PCR products were subcloned into the M13-based vectors M13V<sub>K</sub>PCR3 and M13V<sub>H</sub>PCR1, respectively. Initial nucleotide sequence analysis of  
5 random  $V_L$  region clones revealed that most of the cDNAs encoded the  $V_L$  region of the light chain expressed by the Y3-Ag 1.2.3 rat myeloma cell line (Crowe *et al*, Nucleic Acid Research, 17: 7992, 1989) that was used as the fusion partner to generate the anti-CD4 hybridoma. It is likely  
10 that the expression of the Y3-Ag 1.2.3 light chain mRNA is greater than that of the CD4 antibody light chain, or the Y3-Ag 1.2.3 light chain mRNA is preferentially amplified during the PCR.

To maximize the chance of finding CD4  $V_L$  region cDNAs,  
15 we first screened all M13 clones by hybridisation to a <sup>32</sup>p-labeled oligonucleotide probe that is complementary to the CDR 2 of Y3-Ag 1.2.3 (Crowe *et al*, Nucleic Acid Research, 17: 7992, 1989). Subsequent sequence analysis was restricted to M13 clones which did not contain sequence  
20 complementary to this probe. In this manner, two cDNA clones from independent PCR amplifications were identified that encoded identical  $V_L$  regions. Nucleotide sequence analysis of random  $V_H$  region PCR products revealed a single species of  $V_H$  region cDNA. Two  $V_H$  cDNA clones from  
25 independent PCR amplifications were found to contain identical sequences except that the codon of residue 14 encoded proline [CCT] in one clone while the second clone encoded leucine [CTT] at the same position.

According to Kabat *et al* 1987, 524 of 595 sequenced  $V_H$   
30 regions contain a proline residue at this position, while only 6 contain leucine. We have therefore chosen the proline-encoding clone for illustration (see below). As residue 14 lies well within the first  $V_H$  framework region and not in a CDR, it is unlikely to contribute directly to  
35 antigen binding, and the ambiguity at this position did not

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affect the subsequent reshaping strategy. Thus, we have not investigated this sequence ambiguity further.

The cDNA sequences and their predicted amino acid sequences are shown in Figures 1 and 4. As no additional  
5  $V_L$  or  $V_H$  region-encoding clones were found, it was assumed that these sequences were derived from the CD4 antibody genes.

#### Construction of reshaped antibodies.

Our goal was to investigate the importance of selecting  
10 the appropriate human V region framework during reshaping. Two reshaping strategies were employed.

#### First reshaping strategy.

In the first strategy, we created a reshaped antibody that incorporated the CDRs from the rat-derived CD4  
15 antibody and the same human V region framework sequences that we had previously successfully used for the reshaped CAMPATH-1 antibody, namely an REI-based framework for the  $V_L$  region and an NEW-based framework for the  $V_H$  region (Reichmann *et al*, 1988). This was accomplished by  
20 oligonucleotide-directed in vitro mutagenesis of the six CDRs of the reshaped CAMPATH-1 antibody light and heavy chain cDNAs shown in Figures 2 and 5, respectively. The resultant reshaped CD4 antibody light chain (Figure 3) is called CD4 $V_L$ REI. Two versions of the NEW-based reshaped  
25 CD4 antibody heavy chain were created: CD4 $V_H$ NEW-Thr<sup>30</sup> (Figure 6) encoding a threonine residue at position 30 (in framework 1) and CD4 $V_H$ NEW-Ser<sup>30</sup> (Figure 7) encoding a serine residue at position 30. These two different versions were created because the successfully reshaped  
30 CAMPATH-1 antibody heavy chain bound antigen well whether position 30 encoded a threonine or serine residue

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(Reichmann et al, 1988), and we chose to test both possibilities in this case as well.

### Second reshaping strategy

In the second reshaping strategy, we have reshaped the  
5 CD4 antibody V<sub>H</sub> region to contain the V<sub>H</sub> region framework sequences of the human antibody KOL. Of all known human antibody V<sub>H</sub> regions, the overall amino acid sequence of the V<sub>H</sub> region of KOL is most homologous to the rat CD4 antibody V<sub>H</sub> region. The V<sub>H</sub> regions of the human antibodies KOL and  
10 NEW are 66% and 42% homologous to the rat CD4 antibody V<sub>H</sub> region, respectively.

Two versions of the KOL-based reshaped CD4 antibody heavy chain V region were created that differ by a single amino acid residue within the fourth framework region:  
15 CD4V<sub>H</sub>KOL-Pro<sup>113</sup> (Figure 10) encodes a proline residue at position 113 and CD4V<sub>H</sub>KOL-Thr<sup>113</sup> (Figure 12) encodes a threonine residue at position 113. CD4V<sub>H</sub>KOL-Pro<sup>113</sup> is "true to form" in that its framework sequences are identical to those of the KOL antibody heavy chain V region  
20 (Figure 8).

Of all known human antibody V<sub>L</sub> regions, the overall amino acid sequence of the V<sub>L</sub> region of the human light chain NEW is most homologous (67%) to the rat CD4 antibody V<sub>L</sub> region. Thus, the identical reshaped light chain,  
25 CD4V<sub>L</sub>REI (described above), that was expressed with the NEW-based reshaped CD4 antibody heavy chains CD4V<sub>H</sub>NEW-Thr<sup>30</sup> and CD4V<sub>H</sub>NEW-Ser<sup>30</sup>, is also expressed with the KOL-based reshaped CD4 antibody heavy chains CD4V<sub>H</sub>KOL-Pro<sup>113</sup> and CD4V<sub>H</sub>KOL-Thr<sup>113</sup>. This is advantageous because expression  
30 of the same reshaped light chain with different reshaped heavy chains allows for a direct functional comparison of each reshaped heavy chain.

To summarise, four different reshaped antibodies were created. The reshaped light chain of each antibody is

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called CD4V<sub>L</sub>REI. The reshaped heavy chains of the antibodies are called CD4V<sub>H</sub>NEW-Thr<sup>30</sup>, CD4V<sub>H</sub>NEW-Ser<sup>30</sup>, CD4V<sub>H</sub>KOL-Pro<sup>113</sup>, and CD4V<sub>H</sub>KOL-Thr<sup>113</sup>, respectively. Each of the reshaped heavy chains contain the same human IgG1 constant region. As each reshaped antibody contains the same reshaped light chain, the name of a reshaped antibody's heavy chain shall be used below to refer to the whole antibody (heavy and light chain combination).

Relative affinities of the reshaped antibodies

- 10 The relative affinities of the reshaped antibodies were approximated by measuring their ability to bind to CD4 antigen-expressing cells at various antibody concentrations. FACS analysis determined the mean cellular fluorescence of the stained cells (Table 1).
- 15 It is clear from this analysis that the reshaped CD4 antibodies bind to CD4 antigen to varying degrees over a broad concentration range. Consider Experiment 1 of Table 1 first. Comparing CD4V<sub>H</sub>KOL-Thr<sup>113</sup> antibody to CD4V<sub>H</sub>NEW-Thr<sup>30</sup> antibody, it is clear that both antibodies bind CD4<sup>+</sup> cells when compared to the control, reshaped CAMPATH-1 antibody. However, CD4V<sub>H</sub>KOL-Thr<sup>113</sup> antibody binds CD4<sup>+</sup> cells with far greater affinity than CD4V<sub>H</sub>NEW-Thr<sup>30</sup> antibody. The lowest concentration of CD4V<sub>H</sub>KOL-Thr<sup>113</sup> antibody tested (2.5 ug/ml) gave a mean cellular fluorescence nearly equivalent to that of the highest concentration of CD4V<sub>H</sub>NEW-Thr<sup>30</sup> antibody tested (168 ug/ml). Experiment 2 demonstrates that CD4V<sub>H</sub>NEW-Ser<sup>30</sup> antibody may bind CD4<sup>+</sup> cells somewhat better than CD4V<sub>H</sub>NEW-Thr<sup>30</sup>. Only 2.5 ug/ml CD4V<sub>H</sub>NEW-Ser<sup>30</sup> antibody is required to give a mean cellular fluorescence nearly equivalent to 10 ug/ml CD4V<sub>H</sub>NEW-Thr<sup>30</sup> antibody. Experiment 3 demonstrates that CD4V<sub>H</sub>KOL-Thr<sup>113</sup> antibody may bind CD4<sup>+</sup> cells somewhat better than CD4V<sub>H</sub>KOL-Pro<sup>113</sup> antibody.

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From these assays, it is clear that the KOL-based reshaped antibodies are far superior to the NEW-based reshaped antibodies with regards to affinity towards CD4<sup>+</sup> cells. Also, there is a lesser difference, if any, between

5 CD4V<sub>H</sub>NEW-Thr<sup>30</sup> antibody and CD4V<sub>H</sub>NEW-Ser<sup>30</sup> antibody, and likewise between CD4V<sub>H</sub>KOL-Thr<sup>113</sup> antibody and CD4V<sub>H</sub>KOL-Pro<sup>113</sup> antibody. A ranking of these reshaped antibodies can thus be derived based on their relative affinities for CD4<sup>+</sup> cells:

10 CD4V<sub>H</sub>KOL-Thr<sup>113</sup> > CD4V<sub>H</sub>KOL-Pro<sup>113</sup> >> CD4V<sub>H</sub>NEW-Ser<sup>30</sup> > CD4V<sub>H</sub>NEW-Thr<sup>30</sup>

It should be restated that each of the reshaped CD4 antibodies used in the above experiments have the identical heavy chain constant regions, and are associated with identical reshaped light chains. Thus observed differences

15 of binding to CD4<sup>+</sup> cells must be due to differences in their heavy chain V regions.

Relative avidities of the rat YNB46.1.8 antibody and the reshaped CD4V<sub>H</sub>KOL-Thr<sup>113</sup> antibody

The relative avidities of the rat YNB46.1.8 antibody and

20 the reshaped CD4V<sub>H</sub>KOL-Thr<sup>113</sup> antibody were estimated by ELISA. In this assay, the ability of each antibody to inhibit the binding of biotinylated CD4V<sub>H</sub>KOL-Thr<sup>113</sup> antibody to soluble recombinant CD4 antigen was determined. Results of an experiment are shown in Figure 13. The

25 inhibition of binding of biotinylated CD4V<sub>H</sub>KOL-Thr<sup>113</sup> antibody was linear for both the unlabeled CD4V<sub>H</sub>KOL-Thr<sup>113</sup> and YNB46.1.8 antibodies near the optical density of 0.3. The concentrations of CD4V<sub>H</sub>KOL-Thr<sup>113</sup> and YNB46.1.8 antibodies that give an optical density of 0.3 are 28.7 and

30 1.56 ug/ml, respectively. Thus the avidity of the YNB46.1.8 antibody can be estimated to be 28.7/1.56 or about 18 times better than that of CD4V<sub>H</sub>KOL-Thr<sup>113</sup> antibody. It should be noted that this experiment only provides a rough approximation of relative avidities, not

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affinities. The rat YNB46.1.8 antibody contains a different constant region than that of the CD4V<sub>H</sub>KOL-Thr<sup>113</sup> antibody, and this could affect how well the antibodies bind CD4 antigen, irrespective of their actual affinities for CD4 antigen. The actual affinity of the reshaped antibodies for CD4 antigen may be greater, lesser, or the same as the YNB46.1.8 antibody. The other reshaped antibodies CD4V<sub>H</sub>KOL-Pro<sup>113</sup>, CD4V<sub>H</sub>NEW-Ser<sup>30</sup>, and CD4V<sub>H</sub>NEW-Thr<sup>30</sup> have not yet been tested in this assay.

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Table 1.      Mean cellular fluorescence of CD4<sup>+</sup> cells  
stained with reshaped antibodies

	<u>Reshaped Antibody</u>	<u>Concentration</u> ( $\mu$ g/ml)	<u>Mean cellular</u> <u>Fluorescence</u>
5	<u>Experiment 1.</u>		
	CD4V <sub>H</sub> KOL-Thr <sup>113</sup>	113	578.0
	CD4V <sub>H</sub> KOL-Thr <sup>113</sup>	40	549.0
	CD4V <sub>H</sub> KOL-Thr <sup>113</sup>	10	301.9
10	CD4V <sub>H</sub> KOL-Thr <sup>113</sup>	2.5	100.5
	CD4V <sub>H</sub> NEW-Thr <sup>30</sup>	168	97.0
	CD4V <sub>H</sub> NEW-Thr <sup>30</sup>	40	40.4
	CD4V <sub>H</sub> NEW-Thr <sup>30</sup>	10	18.7
	CD4V <sub>H</sub> NEW-Thr <sup>30</sup>	2.5	10.9
15	CAMPATH-1	100	11.6
	CAMPATH-1	40	9.4
	CAMPATH-1	10	9.0
	CAMPATH-1	2.5	8.6
	CONTROL	----	9.0
20	<u>Experiment 2.</u>		
	CD4V <sub>H</sub> NEW-Thr <sup>30</sup>	168	151.3
	CD4V <sub>H</sub> NEW-Thr <sup>30</sup>	40	81.5
	CD4V <sub>H</sub> NEW-Thr <sup>30</sup>	10	51.0
	CD4V <sub>H</sub> NEW-Thr <sup>30</sup>	2.5	39.3
25	CD4V <sub>H</sub> NEW-Ser <sup>30</sup>	160	260.2
	CD4V <sub>H</sub> NEW-Ser <sup>30</sup>	40	123.5
	CD4V <sub>H</sub> NEW-Ser <sup>30</sup>	10	68.6
	CD4V <sub>H</sub> NEW-Ser <sup>30</sup>	2.5	49.2
	CONTROL	----	35.8
30	<u>Experiment 3.</u>		
	CD4V <sub>H</sub> KOL-Pro <sup>113</sup>	100	594.9
	CD4V <sub>H</sub> KOL-Pro <sup>113</sup>	40	372.0
	CD4V <sub>H</sub> KOL-Pro <sup>113</sup>	10	137.7
	CD4V <sub>H</sub> KOL-Pro <sup>113</sup>	2.5	48.9
35	CD4V <sub>H</sub> KOL-Thr <sup>113</sup>	100	696.7
	CD4V <sub>H</sub> KOL-Thr <sup>113</sup>	40	631.5
	CD4V <sub>H</sub> KOL-Thr <sup>113</sup>	10	304.1
	CD4V <sub>H</sub> KOL-Thr <sup>113</sup>	2.5	104.0
	CONTROL	----	12.3
40			

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CLAIMS

1. A process for the preparation of an antibody chain in which the complementarity determining regions (CDRs) of the variable domain of the antibody chain are  
5 derived from a first mammalian species and the framework of the variable domain and, if present, the or each constant domain of the antibody chain are derived from a second different mammalian species, which process comprises:
  - (i) mutating the framework-encoding regions  
10 of DNA encoding a variable domain of an antibody chain of the said first species such that the mutated framework-encoding regions encode the said framework derived from the said second species; and
  - (ii) expressing the said antibody chain  
15 utilising the mutated DNA from step (i).
2. A process according to claim 1, wherein the framework-encoding regions of DNA encoding the variable domain of an antibody heavy chain are mutated in step (i).
- 20 3. A process according to claim 1 or 2, wherein the framework-encoding regions of DNA encoding the variable domain of an antibody light chain are mutated in step (i).
4. A process according to any one of the preceding claims, wherein the said first species is rat or  
25 mouse.
5. A process according to any one of the preceding claims, wherein the said second species is human.
6. A process according to any one of the preceding claims, comprising:

- 33 -

(a) determining the nucleotide and predicted amino acid sequence of a variable domain of a selected antibody chain of the said first species;

(b) determining the antibody framework to which the framework of the said domain is to be altered;

(c) mutating the framework-encoding regions of DNA encoding the said variable domain such that the mutated framework-encoding regions encode the framework determined upon in step (b).

(d) linking the mutated DNA obtained in step (c) to DNA encoding a constant domain of the said second species and cloning the DNA into an expression vector; and  
(e) introducing the expression vector into a compatible host cell and culturing the host cell under such conditions that antibody chain is expressed.

7. A process according to claim 6, in which about the most homologous framework of an antibody chain of a different species is selected in step (b) as the framework to which the framework of the said variable domain is to be altered.

8. A process according to any one of the preceding claims, wherein the antibody of the said first species is a CD4 antibody.

9. A process according to any one of the preceding claims, wherein the said antibody chain is co-expressed with a complementary antibody chain and antibody comprising the said two chains is recovered.

10. An antibody which is capable of binding to human CD4 antigen, in which the CDRs of the light chain of the antibody have the amino acid sequences:

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CDR1: LASEDIYSDLA

CDR2: NTDTLQN

CDR3: QQYNNYPWT

in which the CDRs of the heavy chain of the antibody have  
5 the amino acid sequences:

CDR1: NYGMA

CDR2: TISHDGSDTYFRDSVKG

CDR3: QGTIAGIRH, and

in which the framework of the variable domain and, if  
10 present, the or each constant domain of each chain are  
derived from a mammalian non-rat species.

11. An antibody according to claim 10, in which  
the mammalian non-rat species is human.

12. An antibody according to claim 11, in which  
15 the variable domain framework of the heavy chain is  
homologous to the heavy chain variable domain framework of  
the protein KOL.

13. An antibody according to claim 12, in which  
the heavy chain variable region has the amino acid sequence  
20 shown in the upper line in Figure 10 or 12.

14. An antibody according to claim 11, in which  
the variable domain framework of the heavy chain is  
homologous to the heavy chain variable domain framework of  
the protein NEW.

25 15. An antibody according to claim 14, in which  
the heavy chain variable region has the amino acid sequence  
shown in the upper line of Figure 6 or 7.

16. An antibody according to any one of claims 11  
to 15, in which the variable domain framework of the light

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chain is homologous to the variable domain framework of the protein REI.

17. An antibody according to claim 16, in which the light chain has the amino acid sequence shown in the  
5 upper line of Figure 3.

18. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, an antibody as claimed in any one of claims 10 to 17.

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## FIG. 1

*HindIII*

```

1  AAGCTTATGAATATGCAAAATCCTCTGAATCTACATGGTAAATATAGGTTTGTCTATACC 59

60  ACAAACAGAAAAACATGAGATCACAGTTCTCTCTACAGTTACTGAGCACACAGGACCTCA 119

-19  M G W S C I I L F L V A T A T -5
120  CCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTAAGGGGTGCA 179

180  CAGTAGCAGGCTTGAGGTCTGGACATATATATGGGTGACAAATGACATCCACTTTGCCCTTT 239

-4      G V H S D I Q L T Q S P V S L S A 13
240  CTCTCCACAGGTGTCCACTCCGACATCCAGCTGACCCAGTCTCCAGTTTCCCTGTCTGCA 299

                                CDR1
14  S L G E T V N I E C L A S E D I Y S D L 33
300  TCTCTGGGAGAACTGTCAACATCGAATGTCTAGCAAGTGAGGACATTTACAGTGATTAA 359

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FIG. 1 (contd.)

34	<u>A</u>	W	Y	Q	Q	K	P	G	K	S	P	Q	L	L	I	Y	CDR 2			53	
360	GCATGGTATCAGCAGAAGCCAGGAAATCTCCTCAACTCCTGATCTATAATACAGATACC																				419
54	<u>L</u>		<u>Q</u>	<u>N</u>	G	V	P	S	R	F	S	G	S	G	T	Q	Y	S	L	73	
420	TTGCCAAATGGGGTCCCTTCACGGTTTAGTGGCAGTGGATCTGGCACACAGTATTCTCTA																	CDR 3			479
74	K	I	N	S	L	Q	S	E	D	V	A	T	Y	F	C	<u>Q</u> <u>Q</u> <u>Y</u> <u>N</u> <u>N</u>			93		
480	AAAATAAACAGCCCTGCAATCTGAAGATGTCGCGACTTATTCTGTCAACAATAACAAT																				539
94	<u>Y</u>		<u>P</u>	<u>W</u>	<u>T</u>	F	G	G	G	T	K	L	E	I	K	R				108	
540	TATCCGTGGACGTTCCGGTGGAGGGACCAAGCTGGAGATCAAAACGTGAGTAGAATTAAAC																				599
BamHI																				620	
600	TTTGCTTCCTCAGTTGGATCC																				620



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## FIG. 2(contd.)

CDR 3

88	C	L	Q	H	I	S	R	P	R	T	F	G	Q	G	T	K	V	E	I	K	107
359	TGCTTGCAGCATATAAGTAGGCGCGCACGTTCCGCCAAGGACCAAGGTGGAATCAAA																				418
108	R	T	V	A	A	P	S	V	F	I	F	P	P	S	D	E	Q	L	K	S	127
419	CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT																				478
128	G	T	A	S	V	V	C	L	L	N	N	F	Y	P	R	E	A	K	V	Q	147
479	GGAAGTGCCTCTGTGTGCTGCTGCTGAATAACTTCTATCCAGAGAGGCCAAAGTACAG																				538
148	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E	S	V	T	E	Q	D	167
539	TGGAAGTGGATAACGCCCTCCAATCGGGTAACCTCCAGGAGAGTGTCTACAGAGCAGGAC																				598
168	S	K	D	S	T	Y	S	L	S	S	T	L	T	L	S	K	A	D	Y	E	187
599	AGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGACTACGAG																				658
188	K	H	K	V	Y	A	C	E	V	T	H	Q	G	L	S	S	P	V	T	K	207
659	AAACACAAAGTCTACGCCCTGCGAAGTCACCCATCAGGCCCTGAGCTCGCCCGTCACAAAG																				
208	S	F	N	R	G	E	C	T	m	HindIII											214
719	AGCTTCAACAGGGGAGAGTGTTAGAAGCTT																				748

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FIG. 3

	HindIII	FIG. 3	M G W S C I	-14
-19	1	AAGCTTGGCTCTACAGTTACTGAGCACACAGACCTCACCATGGGATGGAGCTGTATC		58
-13		I L F L V A T A T G V H S D I Q M T Q S		7
59		ATCCTCTTCTGGTAGCAACAGCTACAGGTGTCCACTCCGACATCCAGATGACCCAGAGC		118
		CDR 1		
8		P S S L S A S V G D R V T I T C <u>L A S E</u>		27
119		CCAAGCAGCCTGAGCGCCAGCGTGGGTGACAGAGTGACCATCACCTGTCTAGCAAGTGAG		178
28		<u>D I Y S D L A</u> W Y Q Q K P G K A P K L L		47
179		GACATTACAGTGATTAGCATGGTACCAGCAGAGCCAGGTAAGGCTCCAAAGCTGCTG		238
		CDR 2		
48		I Y <u>N T D T L Q N</u> G V P S R F S G S G S		67
239		ATCTACAATACAGATACCTTGCAAAATGGTGTGCCAAGCAGATTGAGCGGTAGCGGTAGC		298
68		G T D F T F T I S S L Q P E D I A T Y Y		87
299		GGTACCGACTTCACCTTCACCATCAGCAGCCTCCAGCCAGAGGACATCGCCACCTACTAC		358
		CDR 3		
88		C <u>Q Q Y N N Y P W T</u> F G Q G T K V E I K		107
359		TGCCAACAGTATAACAATTATCCGTGGACGTTGGCCAAAGGACCAAGGTGGAATCAAA		418

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## FIG. 3(contd.)

108	R	T	V	A	A	P	S	V	F	I	F	P	P	S	D	E	Q	L	K	S	127
419	CGAACTGTGGCTGCACCATCTGTCTTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT																				478
128	G	T	A	S	V	V	C	L	L	N	N	F	Y	P	R	E	A	K	V	Q	147
479	GGAAGTGCCTCTGTGTGCTGCTGAATAACTTCTATCCAGAGAGGCCAAAGTACAG																				538
148	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E	S	V	T	E	Q	D	167
539	TGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGAC																				598
168	S	K	D	S	T	Y	S	L	S	S	T	L	T	L	S	K	A	D	Y	E	187
599	AGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAGCAGACTACGAG																				658
188	K	H	K	V	Y	A	C	E	V	T	H	Q	G	L	S	S	P	V	T	K	207
659	AAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAG																				718
208	S	F	N	R	G	E	C	T	m	HindIII											214
719	AGCTTCAACAGGGGAGAGTGTAGAAAGCTT																				748

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## FIG. 4

*HindIII*

```

1  AAGCTTATGAATATGCAAAATCCTCTGAATCTACATGGTAAATATAGTTTGTCTATACC 59

60  ACAAACAGAAAAACATGAGATCAGATTCTCTCTACAGTTACTCAGCACACAGGACCTCA 119

-19  M G W S C I I L F L V A T A T -5
120  CCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTAAGGGGCTCA 179

180  CAGTAGCAGGCTTGAGTCTGGACATATATATGGGTGACAATGACATCCACTTTGCCCTTT 239

-4      G V H S Q V Q L Q E S G G L V Q 13
240  CTCCTCCACAGGTGTCCACTCCAGGTCCAACTGCAGGAGTCTGGTGGAGGCTTAGTGCAG 299

      CDR 1
14  P G R S L K L S C A A S G L T F S N Y G 33
300  CCTGGAAGGTCCTGAAACTCTCTGTGCAGCCTCTGGACTCACTTTCAGTAACTATGGC 359

      CDR 2
34  M A W V R Q A P T K G L E W V A T I S H 53
360  ATGGCCTGGGTCCGCCAGGCTCCAACGAAGGGGCTGGAGTGGTGGCAACCATTAGTCAT 419

```

FIG. 4 (contd.)

54	D	G	S	D	T	Y	F	R	D	S	V	K	G	R	F	T	I	S	R	D	73	
420	<div></div>													GATGGTAGTGACACTTACTTTCGAGACTCCGTTGAAGGGCCGATTCACTATCTCCAGAGAT	479							
74	N	G	K	S	T	L	Y	L	Q	M	D	S	L	R	S	E	D	T	A	T	93	
480	<div></div>													AATGGAAAAGCACCCCTATACCTGCAATGGACAGTCTGAGGTCTGAGGACACGGCCACT	539							
94	Y	Y	C	A	R	<div></div>			Q	G	T	I	A	G	I	R	H	W	G	Q	T	113
540	<div></div>													TATTACTGTGCAAGACAAGGACTATAGCAGGTATACGTCACTGGGGCCAAAGGACCACG	599							
114	V	T	V	S	S	<div></div>													118			
600	<div></div>													GTCACCGTCTCCTCAGGTGAGTCCTTACAACCTCTCTTCTATTCAAGCTTAAATAGATT	659							
660	<div></div>													TTACTGCATTGTGTTGGGGGGAAATGTGTGTATCTGAATTTCAAGTCAATGAAGGACTAGG	719							
720	<div></div>													GACACCTTGGGAGTCAGAAAGGGTCAATTGGGAGCCCCGGGCTGATGCAGACACATCCTC	779							
780	<div></div>													AGCTCCCAGACTTTCATGGCCAGAGATTTATAGGGATCC	817							

BanHI

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BamHI

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Fig. 5

-19	HindIII		M G W S C I I L	-12
1	AAGCTTTACAGT	TACTGACACACAGGACCCTCACCATGGGATGGAGCTGTATCATCCTC		59
-11	F L V A T A T G V H S Q V Q L Q E S G P			9
60	TTCTTGTAGCAACAGCTACAGGTGTCCACTCCCAGGTCCAACCTGCAGGAGCGGTCCA			119
10	G L V R P S Q T L S L T C T V S G F T F			29
120	GGTCTTGAGACCTAGCCAGACCCCTGAGCCTGACCTGCACCGTGTCTGGCTTCACCTTC			179
		CDR 1		
30	T D F Y M N	W V R Q P P G R G L E W I G		49
180	ACCGATTCTACATGAAC	TGGTGAGACAGCCACCTGCAGGCTCTTGAGTGGATTGGA		239
		CDR 2		
50	F I R D K A K G Y T T E Y N P S V K G	R		69
240	TTTATTAGACAAAGCTAAAGGTTACACAACAGAGTACAATCCATCTGTGAAGGGGAGA			299

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## FIG. 5 (contd.)

70 V T M L V D T S K N Q F S L R L S S V T 89  
300 GTGACAAATGCTGTAGACACCAAGCAAGCAAGTTCAGCCTGAGACTCAGCAGCGTGACA 359  
CDR 3  
90 A A D T A V Y Y C A R E G H T A A P F D 109  
360 GCCGCCGACACCGCGTCTATTATTGTGCAAGAGAGGGCCACACTGCTGCTCCTTTGAT 419  
110 Y W G Q G S L V T V S S A S T K G P S V 129  
420 TACTGGGGTCAAGGCAGCCCTCGTCAAGTCTCCTCAGCCTCCACCAAGGCCCATCGGTC 479  
130 F P L A P S S K S T S G G T A A L G C L 149  
480 TTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGCGGGGCACAGCGGCCCTGGGCTGCCTG 539  
150 V K D Y F P E P V T V S W N S G A L T S 169  
540 GTCAAGGACTACTTCCCCGAACCGGTGACGGTGTGCGTGGAACTCAGCGGCCCTGACCAGC 599

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## FIG.5 (contd.)

170 G V H T F P A V L Q S S G L Y S L S S V 189  
600 GCGTGCACACCTTCCCGGCTGTCCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG 659

190 V T V P S S L G T Q T Y I C N V N H K 209  
660 GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAG 719

210 P S N T K V D K K V E P K S C D K T H T 229  
720 CCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCCAAATCTTGTGACAAACTCACACA 779

230 C P P C P A P E L L G G P S V F L F P P 249  
780 TGCCCAACCGTGCCAGCACCTGAACCTCCTGGGGGACCGTCAGTCTTCTCTTCCCCCA 839

250 K P K D T L M I S R T P E V T C V V D 269  
840 AAACCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGAC 899

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## FIG. 5 (contd.)

270 V S H E D P E V K F N W Y V D G V E V H 289  
900 GTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAT 959

290 N A K T K P R E E Q Y N S T Y R V V S V 309  
960 AATGCCAAGACAAAGCCGGGAGGAGCAGTACAACAGCACGTACCGTGTGTCAGCGTC 1019

310 L T V L H Q D W L N G K E Y K C K V S N 329  
1020 CTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTCCAAGGTCTCCAAC 1079

330 K A L P A P I E K T I S K A K G Q P R E 349  
1080 AAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGCGAGCCCCGAGAA 1139

350 P Q V Y T L P P S R D E L T K N Q V S L 369  
1140 CCACAGGTGTACACCTTGCCCCCATCCCGGATGAGCTGACCAAGAACCAGGTACGCCCTG 1199

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FIG. 5(contd.)

370 T C L V K G F Y P S D I A V E W E S N G 389  
1200 ACCTGCCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGAGAGCAATGGG 1259

390 Q P E N N Y K T T P P V L D S D G S F F 409  
1260 CAGCCGGAGAACTACAAGACCACGCCCTCCCGTGGCTGGACTCCGACGGCTCCTTCTTC 1319

410 L Y S K L T V D K S R W Q Q G N V F S C 429  
1320 CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCGGGAACGTCTTCTCATGC 1379

430 S V M H E A L H N H Y T Q K S L S L S P 448  
1380 TCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAGAGCCTCTCCCTGTCTCCG 1439

449 G K Trm *HindIII* 450  
1440 GGTAAATGAGTGGCGACGGGCCCCAAGCTT 1467

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FIG. 6

-19	HindIII		M G W S C I I L	-12
1	AAGCTTTACAGTTACTGAGCACACAGGACCCTCACCATGGGATGGAGCTGTATCATCCTC			59
-11	F L V A T A T G V H S Q V Q L Q E S G P			9
60	TTCTTGGTAGCAACAGCTACAGGTGTCCACTCCAGTCCAACTGCAGGAGCGGTCCA			119
10	G L V R P S Q T L S L T C T V S G F T F			29
120	GGTCTTGAGACCTAGCCAGACCCTGAGCCTGACCTGCACCGTGTCTGGCTTCACCTTC	CDR 1		179
30	T N Y G M A W V R Q P P G R G L E W I G			49
180	ACCAACTATGCCATGGCCTGGGTGAGACAGCCACCTGGACGAGGTCTTGAGTGGATTGGA	CDR 2		239
50	T I S H D G S D T Y F R D S V K G			69
240	ACCATTAGTCATGATGGTAGTGACACTTACTTTCGAGACTCTGTGAAGGGGAGAGTGACA			299

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## FIG. 6 (contd.)

70	M	L	V	D	T	S	K	N	Q	F	S	L	R	L	S	S	V	T	A	A	89
300	ATGCTGGTAGACACCAAGCAAGAACCAAGTTCAGCCTGAGACTCAGCAGCGTGACAGCCGCC																			359	
	CDR 3																				
90	D	T	A	V	Y	Y	C	A	R	Q	G	T	I	A	G	I	R	H	W	G	109
360	GACACCGCGGTCTATTATTGTGCAAGACAAGGCACCTATAGCTGGTATACGTCACTGGGGT																			419	
110	Q	G	S	L	V	T	V	S	S	A	S	T	K	G	P	S	V	F	P	L	129
420	CAAGGCAGCCTCGTCAAGTCTCCTCAGCCTCCACCAAGGCCCATCGGTCTTCCCCCTG																			479	
130	A	P	S	S	K	S	T	S	G	G	T	A	A	L	G	C	L	V	K	D	149
480	GCACCTCCTCCAAGAGCACCTCTGGGGGCCACAGCGGCCCTGGCTGGTCAAGGAC																			539	
150	Y	F	P	E	P	V	T	V	S	W	N	S	G	A	L	T	S	G	V	H	169
540	TACTTCCCCGAACCGGTGACGGTGTGCGTGAAGTCAAGCGGCCCTGACCAAGCGCGGTGCAC																			599	

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## FIG. 6(contd.)

170	T	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S	S	V	V	T	V	189
600	ACCTTCCCGGCTGTCCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG	659																			
190	P	S	S	L	G	T	Q	T	Y	I	C	N	V	N	H	K	P	S	N	209	
660	CCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC	719																			
210	T	K	V	D	K	K	V	E	P	K	S	C	D	K	T	H	T	C	P	P	229
720	ACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCACCG	779																			
230	C	P	A	P	E	L	L	G	G	P	S	V	F	L	F	P	P	K	P	K	249
780	TGCCCAGCACCTGAACCTCCTGGGGGACCGTCAGTCTCTCTTCCCCCAAACCCAAG	839																			
250	D	T	L	M	I	S	R	T	P	E	V	T	C	V	V	D	V	S	H	269	
840	GACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGACGTGAGCCAC	899																			

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## FIG. 6 (contd.)

270	E	D	P	E	V	K	F	N	W	Y	V	D	G	V	E	V	H	N	A	K	289	
900	G	A	G	A	C	C	T	G	A	G	T	C	A	A	C	T	G	T	G	A	C	959
290	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S	V	L	T	V	309	
960	A	C	A	A	G	C	C	G	G	A	G	A	C	A	G	T	A	C	C	G	1019	
310	L	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S	N	K	A	L	329	
1020	C	T	G	C	A	C	C	A	G	G	A	G	T	A	C	A	A	G	T	C	1079	
330	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	349	
1080	C	C	A	G	C	C	C	C	A	T	C	T	C	C	A	A	G	C	C	C	1139	
350	Y	T	L	P	P	S	R	D	E	L	T	K	N	Q	V	S	L	T	C	L	369	
1140	T	A	C	C	C	T	G	C	C	C	C	A	T	G	A	G	T	C	A	A	1199	

FIG. 6 (contd.)

370 V K G F Y P S D I A V E W E S N G Q P E 389  
1200 GTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGAGAGCAATGGGCAGCCGGAG 1259

390 N N Y K T T P P V L D S D G S F F L Y S 409  
1260 AACAACTACAAGACCACGCCCTCCCGTGGACTCCGACGGCTCCTTCTCCTCTACAGC 1319

410 K L T V D K S R W Q Q G N V F S C S V M 429  
1320 AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGAACGCTTCTCATGCTCCGTGATG 1379

430 H E A L H N H Y T Q K S L S L S P G K Trm 448  
1380 CATGAGGCTCTGCACAACCACTACACGCAGAGAGCCTCTCCCTGTCTCCGGTAAATGA 1439

*HindIII*  
1440 GTGCGACGGCCCCAAGCTT 1458

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FIG. 7

-19	HindIII		M G W S C I I L	-12
1	AAGCTTACAGTTACTGAGCACACAGGACCCTCACCATGGGATGGAGCTGTATCATCCTC			59
--11	F L V A T A T G V H S Q V Q L Q E S G P			9
60	TTCTTGGTAGCAACAGCTACAGGTGTCCACTCCCAGGTCCAAGTCAGGAGCGGTCCA			119
10	G L V R P S Q T L S L T C T V S G F T F			29
120	GGTCTTGAGACCTAGCCAGACCCTGAGCCTGACCTGCACCGTGTCTGGCTTACCTTC			179
		CDR 1		
30	S N Y G M A W V R Q P P G R G L E W I G			49
180	AGCAACTATGGCATGGCCCTGGGTGAGACAGCCACCCTGGACGAGGTCTTGAGTGGATTGA			239
		CDR 2		
50	T I S H D G S D T Y F R D S V K G			69
240	ACCATTAGTCATGATGGTAGTGACACTTACTTTCGAGACTCTGTGAAGGGGAGAGTGACA			299

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## FIG. 7 (contd.)

70 M L V D T S K N Q F S L R L S S V T A A 89  
300 ATGCTGGTAGACACCAGCAAGAACCAAGTTTCAGCCTGAGACTCAGCAGCGGTGACAGCCGCC 359  
CDR 3  
90 D T A V Y Y C A R Q G T I A G I R H W G 109  
360 GACACCGCGGTCTATTATTGTGCAAGACAAGGCACTATAGCTGTGTATACGTCACTGGGGT 419  
110 Q G S L V T V S S A S T K G P S V F P L 129  
420 CAAGGCAGCCTCGTCAACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTG 479  
130 A P S S K S T S G G T A A L G C L V K D 149  
480 GCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCCTGGTCAAGGAC 539  
150 Y F P E P V T V S W N S G A L T S G V H 169  
540 TACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGGCCCTGACCAGCGGCGGTGCAC 599

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FIG. 7 (contd.)

170	T F P A V L Q S S G L Y S L S S V T V	189
600	ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG	659
190	P S S L G T Q T Y I C N V N H K P S N	209
660	CCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAAC	719
210	T K V D K K V E P K S C D K T H T C P P	229
720	ACCAAGGTGGACAAGAAAGTTGAGCCCCAAATCTTGTGACAAACTCACACATGCCCAACG	779
230	C P A P E L L G G P S V F L F P P K P K	249
780	TGCCCAGCACCTGAACCTCCTGGGGGACCGTCAGTCTTCCTCTTCCCCCAAACCCAAG	839
250	D T L M I S R T P E V T C V V V D V S H	269
840	GACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGACGTGAGCCAC	899

## FIG .7 (contd.)

270 E D P E V K F N W Y V D G V E V H N A K 289  
900 GAAGACCCCTGAGGTCAAGTTCAACTGTACGTGACGGCGGTGGAGGTGCATAATGCCAAG 959

290 T K P R E E Q Y N S T Y R V V S V L T V 309  
960 ACAAGCCCGGGAGGAGCAGTACAACAGCAGTACCGTGTGGTCAGCGTCCTCACCGTC 1019

310 L H Q D W L N G K E Y K C K V S N K A L 329  
1020 CTGCACCGAGGACTGGCTGAATGGCAAGGAGTACAAGTCAAGGTCTCCAACAAGCCCTC 1079

330 P A P I E K T I S K A K G Q P R E P Q V 349  
1080 CCAGCCCCCATCGAGAAACCATTCTCCAAGCCAAAGGCGAGCCCGAGAACACACAGGTG 1139

350 Y T L P P S R D E L T K N Q V S L T C L 369  
1140 TACACCCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAAGGTACGCCCTGACCTGCCTG 1199

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## FIG. 7 (contd.)

370	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	P	E	389
1200	G	T	C	A	A	G	G	T	T	C	T	A	T	C	C	A	G	C	A	T	1259
390	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L	Y	S	409
1260	A	A	C	A	C	A	G	A	C	C	T	C	C	G	T	G	G	A	C	T	1319
410	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	429
1320	A	A	G	T	C	A	C	C	G	T	G	G	A	C	G	T	C	T	C	T	1379
430	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G	K	T	448
1380	C	A	T	G	A	G	G	T	C	T	C	C	C	T	G	T	C	T	C	C	1439
1440	G	T	G	C	G	A	C	C	C	C	C	A	A	G	C	T	T				1458

*HindIII*

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FIG. 8

1	Q	V	Q	L	V	E	S	G	G	V	V	Q	13								
	CDR 1																				
14	P	G	R	S	L	R	L	S	C	S	S	G	F	I	F	S	S	Y	A	33	
	CDR 2																				
34	M	Y	W	V	R	Q	A	P	G	K	G	L	E	W	V	A	I	I	W	D	53
54	D	G	S	D	Q	H	Y	A	D	S	V	K	G	R	F	T	I	S	R	D	73
74	N	S	K	N	T	L	F	L	Q	M	D	S	L	R	P	E	D	T	G	V	93
	CDR 3																				
94	Y	F	C	A	R	D	G	G	H	G	F	C	S	S	A	S	C	F	G	P	113
114	D	Y	W	G	Q	G	T	P	V	T	V	S	S								126

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## FIG. 9

*HindIII*

```

1  AAGCTTATGAAATGCAAAATCCTCTGAATCTACATGGTAAATATAGTTTGTCTATACC  59

60  ACAAACAGAAAACATGAGATCACAGTTCTCTCTACAGTTACTCAGCACACAGGACCTCA  119

-19  M G W S C I I L F L V A T A T -5
120  CCATGGGATGGAGCTGTATCATCCTCTCTTCTTGGTAGCAACAGCTACAGGTAAGGGCTCA  179

180  CAGTAGCAGGCTTGAGTCTGGACATATATATGGGTGACAAATGACATCCACTTTGCCTTT  239

-4      G V H S Q V Q L V E S G G V Q  13
240  CTCTCCACAGGTGTCCACTCCAGGTCCAACTGGTGGAGTCTGGTGGAGGCGGTGGCAG  299
      CDR1
14  P G R S L R L S C S S S G F I F S N Y G  33
300  CCTGGAAGTCCCTGAGACTCTCCTGTTCCCTCTGCTGATTCATCTTCAGTAACTATGGC  359
      CDR2
34  M A W V R Q A P G K G L E W V A T I S H  53
360  ATGGCCTGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGTGGCGCAACCATTAGTCAT  419

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## FIG. 9 (contd.)

54	D	G	S	D	T	Y	F	R	D	S	V	K	G	R	F	T	I	S	R	D	73
420	GATGGTAGTGACACTTACTTTCGAGACTCCGTTGAAGGCCGATTCACTATCTCCAGAGAT																			479	
74	N	S	K	N	T	L	F	L	Q	M	D	S	L	R	P	E	D	T	G	V	93
480	AATAGCAAAACACCCTATTCTGCAATGGACAGTCTGAGGCCCGAGGACACGGCGGTG																			539	
CDR 3																					
94	Y	F	C	A	R	Q	G	T	I	A	G	I	R	H	W	G	Q	G	T	P	113
540	TATTCTGTGCAAGACAAGGGACTATAGCAGGTATACGTCACTGGGGCCAAAGGACCCCC																			599	
114	V	T	V	S	S																118
600	GTCACCGTCTCCTCAGGTGAGTCCTTACAACCTCTCTTCTTATTCAGCTTAAATAGATT																			659	
660	TTACTGCATTTGTTGGGGGGAATGTGTATCTGAATTCAGGTCATGAAGGACTAGG																			719	
720	GACACCTTGGGAGTCAGAAAGGGTCATTGGGAGCCCGGGCTGATGCAGACAGACATCCTC																			779	
BamHI																					
780	AGCTCCCAGACTTCATGGCCACAGATTTATAGGGATCC																			817	

FIG. 10

-19 HindIII M G W S C I I L F -11  
 1 AAGCTTACAGTTACTCAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTCT 60  
  
 -10 L V A T A T -5  
 61 TCTTGGTAGCAACAGCTACAGGTAAGGGCTCACAGTAGCAGGCTTGAGGTCTGGACATA 120  
  
 -4 G V H S Q V 2  
 121 TATATGGGTGACAATGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCAGGTC 180 27/33  
  
 3 Q L V E S G G V V Q P G R S L R L S C 22  
 181 CAACTGGTGAGTCTGGTGGAGCCGTGGTGCAGCCCTGGAAGGTCCCTGAGACTCTCCTGT 240  
  
 CDR 1  
 23 S S S G F I F S N Y G M A W V R Q A P G 42  
 241 TCCTCCTCTGGATTTCATCTTCAGTAACTATGGCATGGCCCTGGGTCCGCCAGGCTCCAGGC 300  
  
 CDR 2  
 43 K G L E W V A T I S H D G S D T Y F R D 62  
 301 AAGGGGCTGGAGTGGGTGCGCAACCATTAGTCATGATGATGAGTACACTTACTTTCGAGAC 360

FIG. 10 (contd.)

63	<u>S V K G</u>	R F T I S R D N S K N T L F L Q	82
361	TCCGTGAAGGGCCGATTCACTATCTCCAGAGATAATAGCAAAACACCCCTATTCTGCAA		420
		CDR 3	
83	M D S L R P E D T G V Y F C A R <u>Q G T I</u>		102
421	ATGGACAGTCTGAGGCCCGAGGACACGGCGGTATTCTGTGCAAGACAAGGGACTATA		480
103	<u>A G I R H</u> W G Q G T P V T V S S		122
481	GCAGGTATACGTCACCTGGGGCCCAAGGACCCCGTCACCGTCTCCTCAGGTGAGTCCTTA		540
541	CAACCTCTCTTCTATTCAAGCTTAAATAGATTTTACTGCATTGTGGGGGGGAAATGT		600
601	GTGTATCTGAATTTCAGGTCATGAAGGACTAGGACACCTTGGGAGTCAGAAAGGTCAT		660
661	TGGAGCCCCGGCTGATGCAGACAGACATCCTCAGCTCCCAGACTTCATGGCCAGAGATT		720
	<i>Bam</i> HI		
721	TATAGGGATCC		731

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## FIG. 11

*Hind*III

1 AAGCTTATGAATATGCAATCCTCTGAAATCTACATGGTAAATATAGGTTTGTCTATACC 59  
 60 ACAACAGAAAAACATGAGATCAGATTCTCTCTACAGTTACTCAGCACACAGGACCTCA 119  
 -19 M G W S C I I L F L V A T A T -5  
 120 CCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGTAAGGGCTCA 179  
 180 CAGTAGCAGGCTTGAGGCTCGACATATATATGGGTGACAATGACATCCACTTTGCCCTT 239  
 -4 G V H S Q V Q L V E S G G V V Q 13  
 240 CTCGCCACAGGTGTCCACTCCCAGGTCCAACTGGTGGAGTCTGGTGGAGGCGGTGTCAG 299  
 14 P G R S L R L S C S S S G F I F S N Y G 33  
 300 CCTGGAAGGTCCTCGAGACTCTCCTGTTCTCCTCTGGATTTCATCTTCAGTAACATGGC 359  
 34 M A W V R Q A P G K G L E W V A T I S H 53  
 360 ATGGCCTGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTCCGAACCATTAGTCAT 419

CDR 1

CDR 2

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FIG.11 (contd.)

54	D	G	S	D	T	Y	F	R	D	S	V	K	G	R	F	T	I	S	R	D	73			
420	GATGGTAGTGACACTTACTTTCGAGACTCCGTTGAAGGGCCGATTCACTATCTCCAGAGAT																			479				
74	N	S	K	N	T	L	F	L	Q	M	D	S	L	R	P	E	D	T	G	V	93			
480	AATAGCAAAACACCCCTATTCTGCAATGGACAGTCTGAGGCCCGAGGACACGGGCGTG																			539				
94	Y	F	C	A	R	Q				G	T	I	A	G	I	R	H	W	G	Q	G	T	T	113
540	TATTCTGTGCAAGACAAGGGACTATAGCAGGTATACGTCACTGGGGCCAAAGGACCACG																			599				
114	V	T	V	S	S																118			
600	GTCACCGTCTCCTCAGGTGAGTCCTTACAACCTCTCTCTCTTCTATTTCAGCTTAAATAGATT																			659				
660	TACTGCATTGTGGGGGGGAAATGTGTGTATCTGATTTTCAGGTCATGAAGGACTAGG																			719				
720	GACACCTTGGGAGTCAGAAAGGTCATTGGGAGCCCCGGCTGATGCAGACAGACATCCTC																			779				
780	AGCTCCAGACTTCATGCCAGAGATTTATAGGGATCC																			817				

BamHI

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## FIG. 12

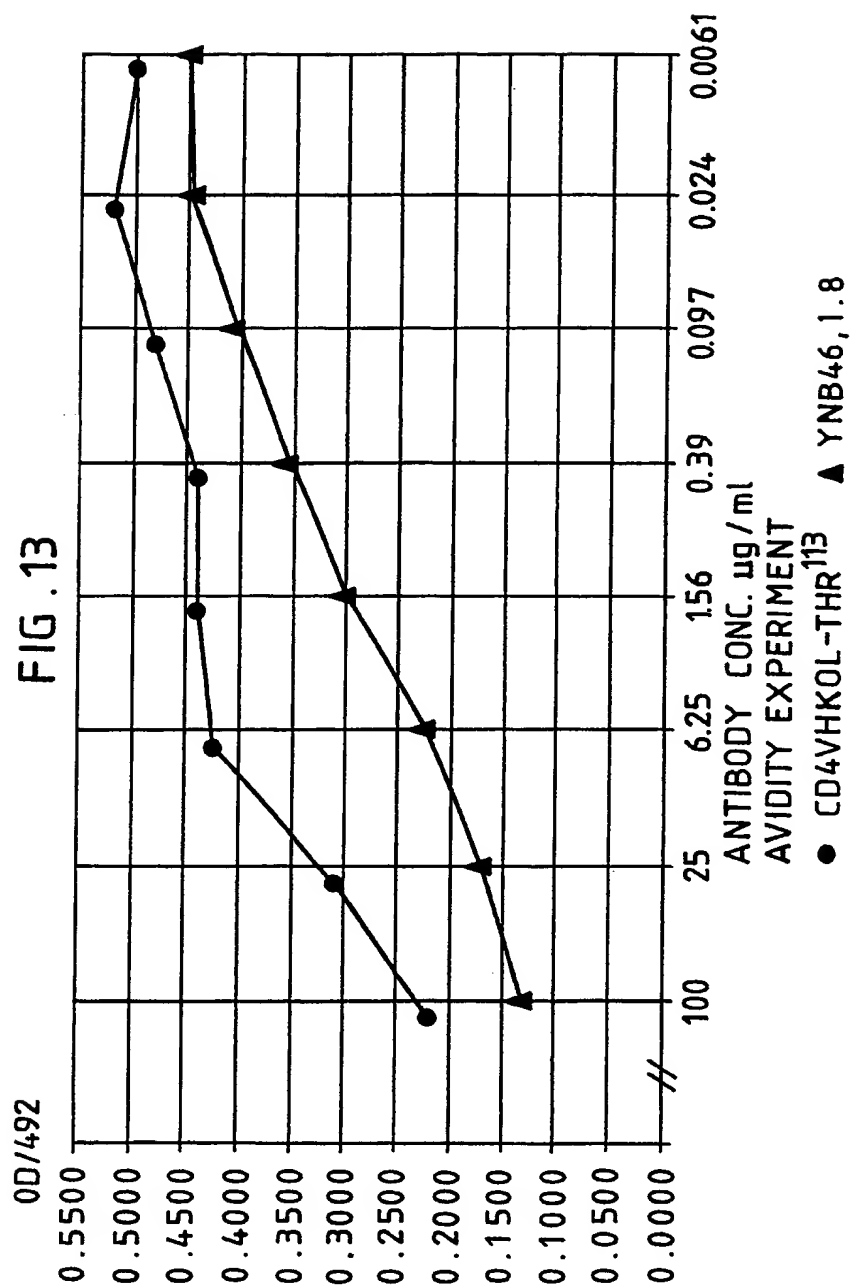
-19 *Hind*III M G W S C I I L F -11  
 1 AAGCTTACAGTTACTCAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTCT 60  
  
 -10 L V A T A T -5  
 61 TCTTGGTAGCAACAGCTACAGGTAGGGGCTCACAGTAGCAGGCTTGAGGTCTGGACATA 120  
  
 -4 G V H S Q V 2  
 121 TATATGGTGACAATGACATCCACTTTGCCCTTTCTCTCCACAGGTGTCCACTCCCAGGTC 180  
  
 3 Q L V E S G G V Q P G R S L R L S C 22  
 181 CAACTGGTGAGTCTGGTGAGGCGGTGGTGCCAGCCTGGAAGGTCCCTGAGACTCTCCTGT 240  
  
 CDR 1  
 23 S S S G F I F S N Y G M A W V R Q A P G 42  
 241 TCCCTCCTCTGGATTTCATCTTCAGTAACTATGGCATGGCCTGGGTCCGCCAGGCTCCAGGC 300  
  
 CDR 2  
 43 K G L E W V A T I S H D C S D T Y F R D 62  
 301 AAGGGCTGGAGTGGTCCGAACCATTAGTCATGATGGTAGTGACACTTACTTTCGAGAC 360  
  
 63 S V K G R F T I S R D N S K N T L F L Q 82  
 361 TCCGTGAAGGCCGATTCACTATCTCCAGAGATAATAGCAAAACACCCATTCTCCTGCAA 420

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FIG. 12 (contd.)

		CDR 3																				
83	M	D	S	L	R	P	E	D	T	G	V	Y	F	C	A	R	<u>Q</u>	<u>G</u>	<u>T</u>	<u>I</u>	102	
421	ATGGACAGTCTGAGGCCCGAGGACACGGCGGTGTATTCTGTGCAAGACAAGGGACTATA																					480
103	<u>A</u>	<u>G</u>	<u>I</u>	<u>R</u>	<u>H</u>	W	G	Q	G	T	T	V	T	V	S	S						122
481	GCAGGTATACGTCACCTGGGCCCAAGGACCACGGTCACCGTCTCCTCAGGTGAGTCCTTA																					540
541	CAACCTCTCTTCTATTTCAGCTTAAATAGATTTTACTGCATTTTGTGGGGGAAATGT																					600
601	GTGTATCTGAATTCAGGTCATGAAGGACTAGGGACACCTTGGGAGTCAGAAAGGTCAT																					660
661	TGGAGCCCCGGCTGATGCAGACAGACATCCTCAGCTCCCAGACTTCATGGCCAGAGATT																					720
BamHI																						
721	TATAGGGATCC																					731

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# INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/01578

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup> According to International Patent Classification (IPC) or to both National Classification and IPC IPC5: C 12 P 21/08, C 12 N 15/13, A 61 K 39/395																							
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Minimum Documentation Searched<sup>7</sup></div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 25%; border: 1px solid black; text-align: left;">Classification System</th> <th style="border: 1px solid black; text-align: left;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; padding: 5px;">IPC5</td> <td style="border: 1px solid black; padding: 5px;">C 12 P; C 12 N; A 61 K</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched<sup>8</sup></div>			Classification System	Classification Symbols	IPC5	C 12 P; C 12 N; A 61 K																	
Classification System	Classification Symbols																						
IPC5	C 12 P; C 12 N; A 61 K																						
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; border: 1px solid black;">Category *</th> <th style="width: 60%; border: 1px solid black;">Citation of Document,<sup>11</sup> with indication, where appropriate, of the relevant passages<sup>12</sup></th> <th style="width: 30%; border: 1px solid black;">Relevant to Claim No.<sup>13</sup></th> </tr> </thead> <tbody> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">X</td> <td style="border: 1px solid black; vertical-align: top;">WO, A1, 9007861 (PROTEIN DESIGN LABS, INC.) 26 July 1990, see page 5; page 10, line 25 - page 14; page 28 - page 30</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-5</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">Y</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">---</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-9</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">X</td> <td style="border: 1px solid black; vertical-align: top;">Proc. Natl. Acad. Sci., vol. 86, December 1989, Cary Queen et al.: "A humanized antibody that binds to the interleukin 2 receptor ", see pages 10029-10033, page 10031 right column-page 10033</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-5</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">Y</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">--</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-9</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">Y</td> <td style="border: 1px solid black; vertical-align: top;">Nature, vol. 341, October 1989, E. Sally Ward et al.: "Binding activities of a repertoire of single immunoglobulin variable domains secreted from Escherichia coli ", see page 544 - page 546</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-9</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;"></td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">---</td> <td style="border: 1px solid black;"></td> </tr> </tbody> </table>			Category *	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>	X	WO, A1, 9007861 (PROTEIN DESIGN LABS, INC.) 26 July 1990, see page 5; page 10, line 25 - page 14; page 28 - page 30	1-5	Y	---	1-9	X	Proc. Natl. Acad. Sci., vol. 86, December 1989, Cary Queen et al.: "A humanized antibody that binds to the interleukin 2 receptor ", see pages 10029-10033, page 10031 right column-page 10033	1-5	Y	--	1-9	Y	Nature, vol. 341, October 1989, E. Sally Ward et al.: "Binding activities of a repertoire of single immunoglobulin variable domains secreted from Escherichia coli ", see page 544 - page 546	1-9		---	
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Y	---	1-9																					
X	Proc. Natl. Acad. Sci., vol. 86, December 1989, Cary Queen et al.: "A humanized antibody that binds to the interleukin 2 receptor ", see pages 10029-10033, page 10031 right column-page 10033	1-5																					
Y	--	1-9																					
Y	Nature, vol. 341, October 1989, E. Sally Ward et al.: "Binding activities of a repertoire of single immunoglobulin variable domains secreted from Escherichia coli ", see page 544 - page 546	1-9																					
	---																						
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>																							
<b>IV. CERTIFICATION</b> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border: 1px solid black; padding: 5px;">           Date of the Actual Completion of the International Search             16th December 1991         </td> <td style="width: 50%; border: 1px solid black; padding: 5px;">           Date of Mailing of this International Search Report   <div style="text-align: center;">08 JAN 1992</div> </td> </tr> <tr> <td style="border: 1px solid black; padding: 5px;">           International Searching Authority             EUROPEAN PATENT OFFICE         </td> <td style="border: 1px solid black; padding: 5px;">           Signature of Authorized Officer   <div style="text-align: center;">MISS T. TAZELAAR</div> </td> </tr> </table>			Date of the Actual Completion of the International Search  16th December 1991	Date of Mailing of this International Search Report  <div style="text-align: center;">08 JAN 1992</div>	International Searching Authority  EUROPEAN PATENT OFFICE	Signature of Authorized Officer  <div style="text-align: center;">MISS T. TAZELAAR</div>																	
Date of the Actual Completion of the International Search  16th December 1991	Date of Mailing of this International Search Report  <div style="text-align: center;">08 JAN 1992</div>																						
International Searching Authority  EUROPEAN PATENT OFFICE	Signature of Authorized Officer  <div style="text-align: center;">MISS T. TAZELAAR</div>																						

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Nature, vol. 332, March 1988, L Riechmann et al.: "Reshaping human antibodies for therapy", see page 323 - page 327 page 526 right column --	1-9
X	EP, A1, 0328404 (MEDICAL RESEARCH COUNCIL) 16 August 1989, see page 4; page 9, line 30; page 11, line 5 ---	1-5
X	EP, A2, 0365209 (BECTON DICKINSON AND COMPANY) 25 April 1990, see in particular col. 3, lines 27-49 and columns 5-8 --	1-5
A	Proc.Natl.Acad.Sci., vol. 87, June 1990, J Sharon: "Structural correlates of high antibody affinity: Three engineered amino acid substitutions can increase the affinity of an anti-p-azophenylarsonate antibody 200-fold", see page 4814 - page 4817 --	1
A	Science, vol. 239, March 1988, M Verhoeven et al.: "Reshaping Human Antibodies: Grafting an Antilysozyme Activity", see page 1534 - page 1536 --	1-9
A	Nature, vol. 321, May 1986, P T Jones et al.: "Replacing the complementarity-determining regions in a human antibody with those from a mouse", see page 522 - page 525 page 525, left column --	1
A	Nature, vol. 328, August 1987, S. Roberts et al.: "Generation of an antibody with enhanced affinity and specificity for its antigen by protein engineering", see page 731 - page 734 ---	1

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,X	WO, A1, 9109966 (ORTHO PHARMACEUTICAL CORPORATION) 11 July 1991, see the whole document  --	1-5
P,X	WO, A1, 9107492 (CENTRAL BLOOD LABORATORIES AUTHORITY) 30 May 1991, see page 3  --	1
P,X	EP, A1, 0403156 (GENZYME CORPORATION) 19 December 1990, see example 12  --	1-5
P,X	WO, A1, 9109967 (CELLTECH LIMITED) 11 July 1991, see the whole document  --  -----	1-9

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO. PCT/GB 91/01578**

SA 51310

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9007861	26/07/90	AU-D- 5153290	13/08/90
		CA-A- 2006865	28/06/90
		EP-A- 0451216	16/10/91
EP-A1- 0328404	16/08/89	AU-D- 3062689	06/09/89
		GB-A- 2216126	04/10/89
		WO-A- 89/07452	24/08/89
EP-A2- 0365209	25/04/90	JP-A- 2238883	21/09/90
WO-A1- 9109966	11/07/91	WO-A- 91/09967	11/07/91
		WO-A- 91/09968	11/07/91
WO-A1- 9107492	30/05/91	AU-D- 6721490	13/06/91
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